Relationship between the nemertean *Cerebratulus lacteus* and the soft-shell clam, *Mya arenaria*: Predator behaviour and prey selection.

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Abstract

Several studies have shown that the nemertean *Cerebratulus lacteus* is an important predator in soft-shell clam (*Mya arenaria*) populations. The presence of this predator in culture sites is raising some concerns from clam farmers. A laboratory study was undertaken in 1999 in Prince Edward Island to better describe the predatory behaviour of *C. lacteus*. Experiments carried out addressed 1) prey species selection, 2) size selection in soft-shell clams, 3) predator density, 4) quality of clams, and 5) polychaete addition as a control measure. Results showed that *C. lacteus* preferred soft-shell clams. No other species displayed any signs of predation. *C. lacteus* showed no size preference for soft-shell clams as well. High predator densities did not impede predation on clams, mortality rose with increasing density within a 3 week period. Prey selection on clams did not vary in relation to their quality (healthy versus sick clams). Finally, the polychaete addition experiment showed that *Nereis virens* was an ineffective predator control measure. *Glycera dibranchiata*, however, showed promising results by significantly reducing clam mortality in the presence of *C. lacteus* by 13 %. There was, however, a 9% mortality of clams attributed to the presence of this polychaete.

Résumé

De nombreuses études ont démontré que le némerte *Cerebratulus lacteus* est un prédateur important au sein des bancs de myes (*Mya arenaria*) en milieu naturel. La présence de ce prédateur sur les sites de culture de myes soulève présentement beaucoup d'inquiétudes de la part des cultivateurs. Des études expérimentales ont été entreprises en laboratoire en 1999 à l'Île-du-Prince-Édouard afin de mieux comprendre le comportement de prédation de *C. lacteus*. Ces études cherchaient à préciser 1) la sélection de proies, ainsi que 2) la sélection de taille de myes et à étudier l'effet de 3) la densité du prédateur, 4) la condition physiologique de myes, et 5)

l'addition de polychètes sur le comportement de prédation de *C. lacteus*. *C. lacteus* a démontré une préférence significative envers les myes peu importe la taille de celles-ci. Aucune des autres espèces de bivalve n'a subit de prédation. L'augmentation de la densité de *C. lacteus* n'a montré aucun signe de ralentissement de la prédation sur les myes à l'intérieur d'une période de 3 semaines. Nos résultats suggèrent également que le taux de prédation de *C. lacteus* ne varie pas en fonction de la qualité des myes. Finalement, l'addition du polychète *Nereis virens* ne s'est pas avérée une bonne mesure de contrôle du prédateur. La présence de *Glycera dibranchiata* a réduit significativement le taux de prédation de *C. lacteus*. Une mortalité de myes de 9 % a cependant été observé en présence de ce polychète.

Introduction

Over the last decade, aquaculture activities in Prince Edward Island have shown an increasing interest in cultivating soft-shell clams (*Mya arenaria*). The presence of the nemertean *Cerebratulus lacteus* in culture sites has raised some concerns from clam farmers. Prouse et al. (1988) have partially attributed high soft-shell clam collapses to the presence of this worm in the Annapolis Basin in Nova Scotia. Rowell and Woo (1990) later observed experimentally, in the same area and in laboratory, that *C. lacteus* was a true predator of the soft-shell clams. Bourque et al. (1999) recently confirmed that *C. lacteus* was an effective predator of soft-shell clams in Prince Edward Island's culture sites. Mortality rates of soft-shell clams monitored in laboratory assays reached 100% in a few weeks in the presence of *C. lacteus* compared to 0% in its absence. Bourque et al. (1999) also tested, in parallel, field and laboratory sediment manipulations and laboratory polychaete additions as possible control measures for the soft-shell clam industry. These measures were proven ineffective.

The biology of *C. lacteus* is still poorly known and behavioural information is often anecdotal. McDermott (1976), for instance, described the predation of *C. lacteus* on razor clam *Ensis directus* in Cheasapeake Bay. Landry et al. (1993) later showed a possible link between high field mortalities of the quahog *Mercenaria mercenaria* and the presence of *C. lacteus* on Prince Edward Island's sandflats. It is not known if the quality of prey has any effect on the predation behaviour of *C. lacteus*. Many studies have shown that *C. lacteus* are distributed in patches, and that densities can reach over 20 ind./m^2 (Wilson 1900, Rowell and Woo 1990 and Bourque et al. 1999). Again, no information on the effects of this gregarious behaviour is available in order to determine if density impedes or enhances predation on soft-shell clam populations. The aim of this study is to better describe the predation behaviour of *C. lacteus*, and

to confirm several tendancies that were observed in a preliminairy study on its relationship with *M. arenaria* (Bourque et al. 1999). More precisely, experiments were designed to address 1) preferred bivalve prey-species and 2) preferred size-classes in soft-shell clams as well as the effect of 3) predator density, 4) prey quality (healthy versus sick clams), and polychaete presence on the predation rate of *C. lacteus*. A better understanding of the predation behaviour would help in the development of effective nemertean control measures for the soft-shell clam industry.

Material and methods

Experimental set-up

Laboratory studies were undertaken at Ellerslie hatchery, Prince Edward Island, between June and August 1999. The experimental set-up was the same as the one described by Bourque *et al.* (1999). The studies were conducted in plastic aquaria containing a layer of sediments approximately 10 cm deep. Sediments were sieved on site (Port Hill) with a 1 mm mesh. The aquaria were supplied with sea water via a parallel flow-through system. The water was pumped from the Bideford River (estuary) via a sand filter. The mean temperature and salinity were maintained at 21°C and 20‰ respectively throughout the study period. The water flow was adjusted with small clamps. The aquaria were placed in larger containers, which served as collecting bins for water overflow and any escaping organisms. A hole was cut out of the bottom of the plastic bins to serve as drains. A rubber stopper with an elevated drain tube was inserted into the hole. The extremity of the tube was covered with a 2 mm mesh to prevent organisms from escaping. PVC tubing (15 cm diameter) was used to support all of the plastic bins and serve as a drainway. Photoperiod was maintained at 8 hours of light and 16 hours of darkness.

All aquaria used in the following studies were set up as described above. *C. lacteus* used in all of these studies were obtained from West River because of their occurrence in high densities.

Prey selection

Prey selection was studied by providing *C. lacteus* with the choice of four bivalve species often found in nemertean habitats (*M. arenaria*, *Mercenaria mercenaria*, *Crassostrea virginica* and *Mytilus edulis*). *M. arenaria* (37.52 ± 0.76 mm; n = 51) were obtained from Bedeque Bay (Miscouche), *M. mercenaria* (46.32 ± 0.51 mm; n = 51) and *C. virginica* (106.88 ± 2.44 mm; n = 51) were purchased from a Malpeque fisherman, and *M. edulis* (65.42 ± 0.94 mm; n = 51) were collected from Bideford River. Prey were feed indirectly through the water source. Five replicates were used for each of the treatments. Predator treatments were conducted in 38 cm X 25 cm aquaria. Control treatment (no predator) consisted of two 38 cm X 25 cm aquaria and three 30 cm X 15 cm aquaria. Each aquarium contained 6 individuals of each species, while the smaller aquaria held 3 individuals of each species. These steps were taken in order to obtain similar densities in all aquaria. Three *C. lacteus* were introduced into predator treatment aquaria. After one week the aquaria were emptied and bivalve mortalities, if any, were recorded.

Clam size selection

A series of ten aquaria (38 cm X 25cm) were set up to confirm results from a preliminary clam size selection experiment conducted in 1998. Five replicates were used for each treatment (absence and presence of predator). Clams were obtained from Bedeque Bay (Miscouche) and divided into three size classes: 0-15 mm; 15-50 mm; >50 mm. Eight clams from each of the three size classes were placed in each of the 10 aquaria for a total of 24 individuals per aquarium.

Three *C. lacteus* were introduced into 5 aquaria. All aquaria were emptied after 2 weeks and the mortality rate of clams was determined.

Predator density

The effect of predator density on clam mortality was studied. Clams ranging from 15 to 50 mm in size were obtained from Darnley Basin. Four *C. lacteus* density classes were studied: 0 (control), 1, 3 and 6 individuals per aquarium (0, 11, 33 and 66 ind./m^2). For each density class, 9 replicates were used (total of 36 aquaria). The aquaria measured 36 cm X 25 cm, except for the control aquaria which were 30 cm X 15 cm in size. Large aquaria contained 24 soft-shell clams, while the smaller ones housed 12 individuals. After the first week, three aquaria for each density class were emptied to evaluate clam mortality. The same procedure was done after 2 and 3 weeks to follow mortality rate in time.

Clam health and predation

A study of predation susceptibility was initiated after high mortalities were observed in control treatments from the predator density study. A large number of clams from Darnley Basin were diagnosed with a blood disease, hematoneoplasia (McGladdery, personal communication), which was confirmed through standard histological methods. Healthy clams (showing no signs of illness) were collected from Bedeque Bay (Miscouche). Five treatments in triplicate were studied: 1) healthy clams (control), 2) sick clams (control), 3) healthy clams with predators, 4) sick clams with predators, and 5) healthy and sick clams with predators (same aquarium). Sick clams were identifiable by shrivelled siphons and blisters. In treatment 5, sick clams were distinguished from healthy ones by marking the shells in black. Clam densities were defined as

24 individuals per aquarium. Aquaria with predators contained three *C. lacteus*. Aquaria were emptied two weeks after experiments started to evaluate clam mortality.

Polychaete addition

The predation on *M. arenaria* by *C. lacteus* was evaluated in the presence of two polychaete species used as a biological control measure, *Nereis virens* and *Glycera dibranchiata*. Eleven species combinations were studied: *C. lacteus*, *M. arenaria* and *N. virens* (C /M/N); *C. lacteus*, *G. dibranchiata* and *M. arenaria* (C/G/M); *C. lacteus* and *N. virens* (C/N); *C. lacteus* and *M. arenaria* (C/M); *C. lacteus* and *G. dibranchiata* (C/G); *N. virens* and *M. arenaria* (N/M); *G. dibranchiata* and *M. arenaria* (G/M); *C. lacteus* (C); *M. arenaria* (M); *N. virens* (N); *G. dibranchiata* (G). Each species combination was studied in triplicate. Polychaetes and clams were obtained from West River. The number of animals for each species used in each aquaria are presented in Table 1. Nemerteans were weighed (towelled fresh weight) before and after the trials in grams with a precision of three decimals. The collecting bins were regularly checked for migrating organisms which were quantified. Aquaria were emptied after 19 days, when approximately 50% mortality was thought to be attained, and clam mortality was recorded.

Statistical analysis

The data were analysed with Systat 6.0© for Windows©. All analyses were calculated at a confidence level of $\alpha = 0.05$. Prey selection did not require any statistical analysis because of obvious results. Clam size selection was analysed using a two-factor ANOVA. The factors were size-class and presence or absence of predator. Non-parametric analysis of variance were used when normality was not attainable through transformations: 1) predator density data were analysed with a Kruskal-Wallis two-factor analysis of variance, involving density and number of

weeks; 2) clam health and 3) polychaete addition were analyzed with a Kruskal-Wallis one-way analysis of variance. Clam size selection was analysed with a two-factor ANOVA.

Results

Statistical analysis were not required in the prey selection study because of very convincing results. Only *M. arenaria* displayed mortality over 50% after one week in the presence of *C. lacteus* (Fig. 1). All other prey species showed 0% mortality regardless of the presence or absence of the predator.

The ANOVA showed a significant effect of size-class ($F_{2,24}$=5.283; P=0.013) and treatments ($F_{1,24}$=8.696; P=0.007) as well as an interaction effect ($F_{2,24}$=4.717; P=0.019) between size-classes and treatments. Mortality of clams in predator treatments for all clam size-classes were high (between 40 % and 50 %). Clam mortality in the 0-15 mm size-class was also considerable (≈50%) in the control treatment. Control treatments of the 15-50 mm and >50 mm size-classes displayed much lower clam mortality (Fig. 2). Clam mortality doubled in the 15-50 mm and >50 mm size-classes with predators.

Results from the predator density study indicated that density had a significant effect on the mortality of soft-shell clams ($H_{3,24}$=22.018; P< 0.001). Clam mortality rose considerably with increasing densities of *C. lacteus* (Fig. 3). The Tukey multiple comparisons test did not reveal any significant differences between densities 0 and 1, 1 and 3, and 3 and 6 *C. lacteus* per aquaria. After one week, clam mortality increased very little between densities of *C. lacteus*. However, there is a slight increase of clam mortality in the 6 *C. lacteus* per aquaria density. Although not significant, the time factor ($H_{2,24}$=3.765; 0.10<P<0.25), revealed slight tendencies within densities. Clam mortality in aquaria without *C. lacteus* was approximately 10% for all three

weeks. There was a slight increase in clam mortality at a density of 1 *C. lacteus* between the first and second week, and no increase between the second and third week. Densities of 3 and 6 *C. lacteus* showed important levels of clam mortality for each week. No interaction between the two factors ($H_{6,24}$=12.592; $0.75 < P < 0.9$) was observed in clam mortality rates. Clam mortality rate per *C. lacteus* decreased with increasing densities of *C. lacteus* (Fig. 4). There was a drop in mortality between densities of 1 and 3 *C. lacteus* for all three weeks. While clam mortality was stable between densities of 3 and 6 *C. lacteus*. Predation rate was highest in 1 *C. lacteus* density with 0.45 clams/predator/day. Predation rates for 3 and 6 C. lacteus were slightly lower at 0.2 clams/predation/day.

The Kruskal-Wallis analysis on healthy and sick clams showed a significant difference between the treatments studied ($H_{5,12}$=13.111; P=0.021) (Fig. 5). Tukey multiple comparison analysis showed that the healthy clam control was significantly different from healthy clam with the predator treatment. No other differences were revealed between the other treatments. The sick clam control treatment showed 15 % mortality, while healthy clams showed no mortality. Healthy clams with predator showed a slightly higher clam mortality (49%) than sick clams with predator (44%).

Analysis of clam mortality with the addition of polychaetes showed significant differences among treatments ($H_{5,12}$=14.987; P=0.01). Highest clam mortalities were associated with the presence of *C. lacteus*. The control treatment (M) showed 0 % mortality. The addition of *N. virens* did not significantly decrease clam mortality due to the presence of *C. lacteus*. Clam mortality was significantly reduced in the C/G/M treatment by 13% (Fig. 6). A 9 % mortality was, however, observed in the G/M treatment. Mortality of *N. virens* varied between 0 and 10 %. Variations between treatments were not significant ($H_{3,8}$=6.351; P=0.096). *N. virens*

migrated out of aquaria in the presence of *C. lacteus*. This response, however, was highly variable within treatments between 0 and 40 % ($H_{3,8}$=3.194; P=0.363) (Fig. 7). The analysis of weight variation in *C. lacteus* showed important weight gain when *M. arenaria* was present. Treatments C/N and C showed weight loss (Fig. 8).

Discussion

Prey selection amongst the four bivalve species tested was evident after only one week. The bivalve species that were chosen occupy two different habitats (endobenthic versus epibenthic habitats). *C. lacteus* may possibly only prey upon endobenthic species, thus eliminating the possibility of preying upon the epibenthic *C. virginica* and *M. edulis*. In this scenario, only *M. arenaria* and *M. mercenaria* would be susceptible to predation. *M. mercenaria* is capable of closing its valves firmly, which in turn would make predation by *C. lacteus* more difficult. *M. arenaria*, however, is not able to close its valves tightly which makes it an easier prey. *C. lacteus* apparently targets the exposed flesh such as the siphon (Bourque, personal observations). If predation by *C. lacteus* is not restricted to endobenthic species, chances are that epibenthic species would be harder to find for this endobenthic predator. Between these four bivalve species, *M. arenaria* is the species most likely to be affected by this predator in the natural environment. The size factor for *C. virginica* cannot be factored out, but the epibenthic habitat most likely has a greater impact on selection. Further studies should focus on the possibility of predation on the other species when *M. arenaria* is not present, particularly *M. mercenaria* and *Ensis directus*.

Clam size selection results revealed high mortality in all size-classes in predator treatments. Clam mortality in the 0-15 mm size-class control treatment was over 40%. The

excessive mortality detected in this treatment (no predators present) could be attributed to reduced survival of this size-class in the experimental aquaria. This mortality most probably explains the interaction effect observed between treatments and size-classes on clam mortality rates. There was no increase in mortality for the 0-15 mm size-class when *C. lacteus* was present, which suggests little or no selection of this predator. This could indicate size selection toward larger clams. The 0-15 mm size-class could also be less favoured because their small size would make them more difficult to find if *C. lacteus* is a random predator. These results are consistent with observations reported by Bourque *et al.* (1999).

Various densities of *C. lacteus* affected the mortality rate of soft-shell clams. Total clam mortality rose with increasing densities of *C. lacteus*. Mortality remained stable for the first week, and this may be explained by an acclimation period of the nemerteans. The slight increase in mortality observed in the density of 6 *C. lacteus* during the first week could be attributed to individual variations of acclimation. The time factor, although not statistically significant, revealed some interesting results. Aquaria without *C. lacteus* showed 10 % mortality which was later attributed to hematoneoplasia. Clam mortality, therefore, is most likely overestimated in the other *C. lacteus* densities. Clam mortality at a density of 1 *C. lacteus* remained stable between the second and third week, which suggests a possible time lapse between nemertean feedings. This time laps could be hidden at higher densities because of feeding time variations in individuals. Clam mortality per *C. lacteus* would be expected to remain stable if *C. lacteus* density had an additive effect on clam mortality. This is the case between densities of 3 and 6 *C. lacteus*. An important decrease was observed between densities of 1 and 3 *C. lacteus*, and could indicate intraspecific competition. A similar trend, however, should have also been observed at higher densities.

Sick clams did not display greater mortality in the presence of *C. lacteus* than healthy clams. Nevertheless, a 15 % mortality in the sick clam control treatment indicates that predation on sick clams is possibly overestimated. If this is true, then healthy clams (HCP) suffered greater predation than sick clams (SCP). Results from the healthy and sick clams with predator (HSCP) treatment, when considering sick clam "natural" mortality, revealed equal predation on clams regardless of their health. This indicates that predation by *C. lacteus* most likely occurs upon finding a soft-shell clam without consideration for its physiological condition.

The use of polychaetes as a predation control method showed some interesting results. *N. virens* was confirmed as being ineffective in controlling nemertean predation (Bourque et al. 1999). Mortality of *N. virens* was highest in the C/N treatment (≈40%), which may indicate predation by *C. lacteus*. However, analysis of nemertean weight showed a decrease in the same treatment, indicating either absence of predation, poor quality of *N. virens* as a prey or competition between these species. Migration of *N. virens* was much less pronounced than results found by Bourque *et al.* (1999), and did not show any significant increase when *C. lacteus* was present. The presence of *G. dibranchiata* significantly reduced the predation rate of *C. lacteus* on clams, however, there was a 9% clam mortality when *G. dibranchiata* was placed alone with the clams. Therefore, clam mortality caused by *C. lacteus* is possibly overestimated in the C/G/M treatment due to the presence of *G. dibranchiata*.

Conclusion

Soft-shell clams are the preferred prey of *C. lacteus* when given a choice between four common bivalve species. *C. lacteus* tend to select clams larger than 15 mm. The mortality rate of clams increased with *C. lacteus* densities, however, mortality per individual decreased

between densities of 1 and 3 *C. lacteus*. Sick clams were not more susceptible to predation by *C. lacteus*. The addition of *N. virens* was proven to be ineffective in reducing predation on clams. *N. virens* also showed a negative response to the presence of *C. lacteus*, although there was no indication of predation of *N. virens* by *C. lacteus*. The preference of soft-shell clams, size selection and no selection of physiological condition by *C. lacteus* coupled with its gregarious behaviour makes it an important threat to natural and cultivated clam populations. Further studies are needed to better understand the mechanisms involved in the predation behaviour of *C. lacteus*, and to develop effective control measures for aquaculture purposes.

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Table 1. Species combinations and number of individuals used in aquaria in the polychaete addition experiment.

Treatment	Species	Number of individuals
C/M/N	*C. lacteus*	1
	M. arenaria	25
	N. virens	10
C/N	*C. lacteus*	1
	N. virens	10
N/M	*N. virens*	6
	M. arenaria	14
C/M	*C. lacteus*	1
	M. arenaria	25
N	*N. virens*	6
C	*C. lacteus*	1
M	*M. arenaria*	14
G	*G. dibranchiata*	5
C/G/M	*C. lacteus*	1
	G. dibranchiata	10
	M. arenaria	25
C/G	*C. lacteus*	1
	G. dibranchiata	10
G/M	*G. dibranchiata*	5
	M. arenaria	12

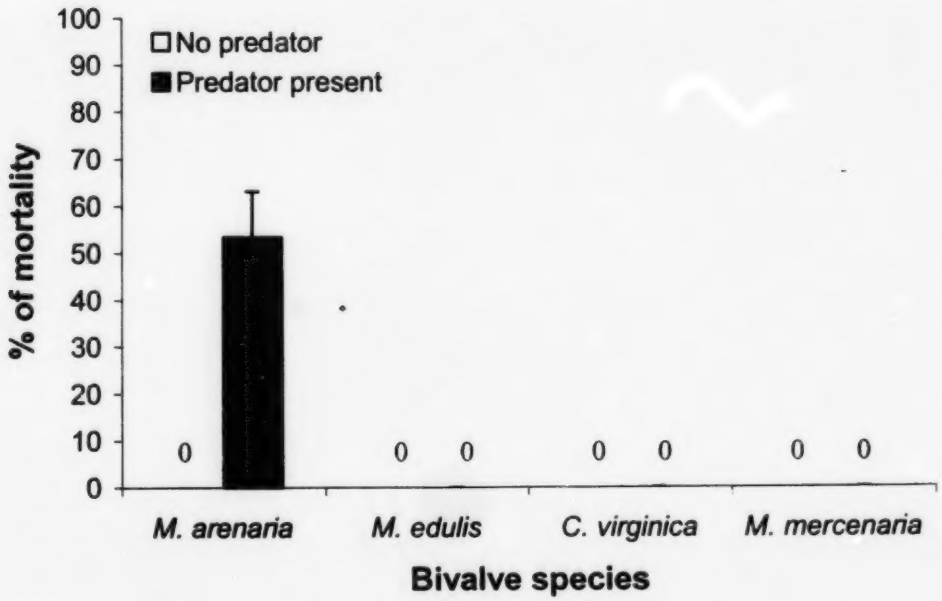

Figure 1. Percentage (mean ± SE; n = 5) of mortality of four bivalve species in the presence or absence of *Cerebratulus lacteus*.

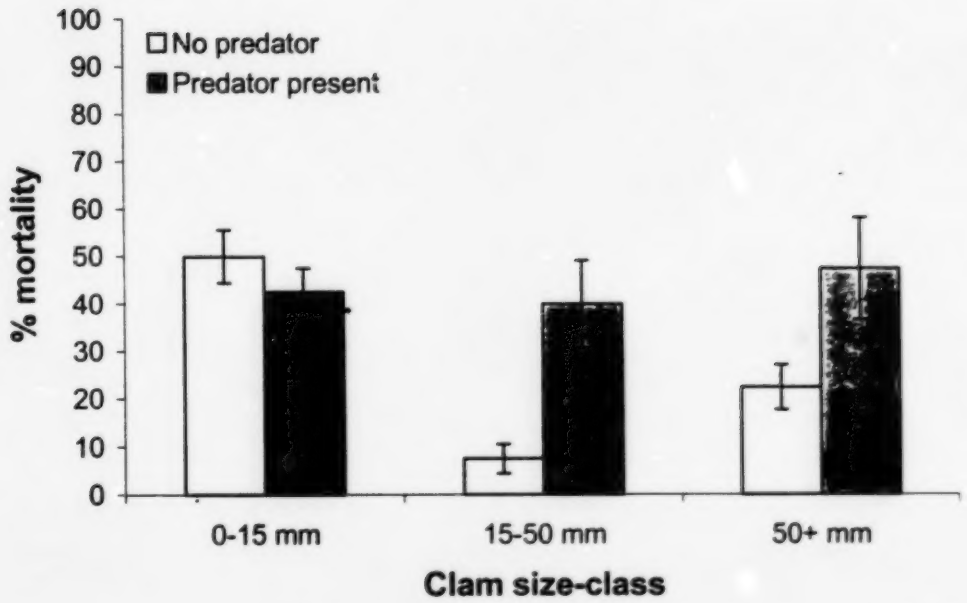

Figure 2. Percentage (mean ± SE; n = 5) of mortality per size-class of *Mya arenaria* in presence or absence of predator, *Cerebratulus lacteus*.

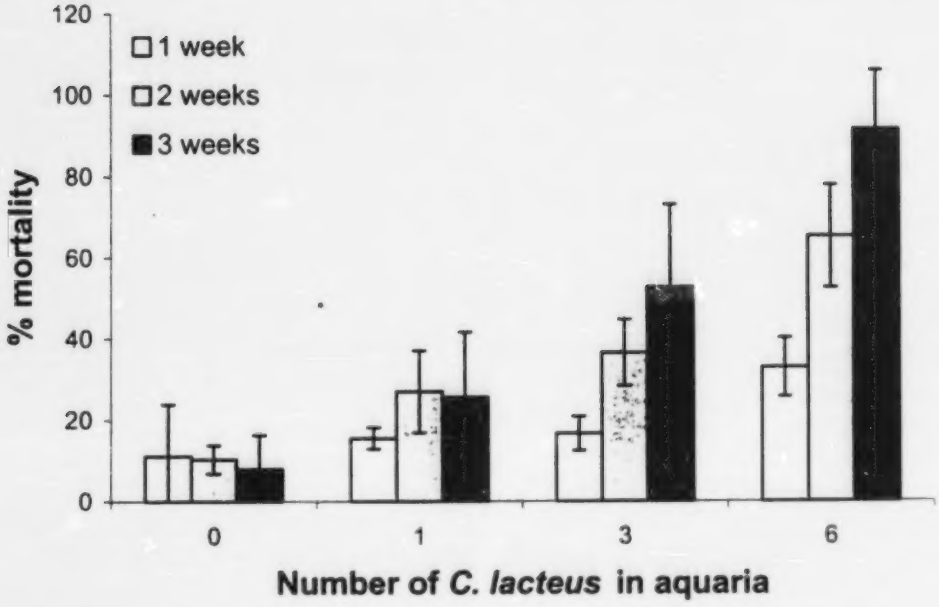

Figure 3. Percentage (mean ± SE; n = 3) of mortality of *Mya arenaria* in relation to predator density after 1, 2 and 3 weeks.

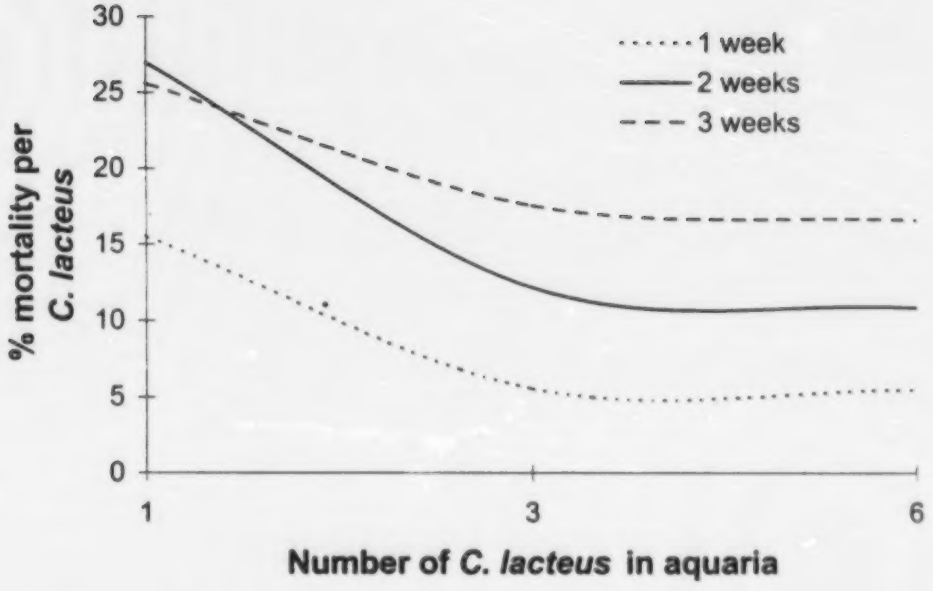

Figure 4. Percentage (mean ± SE; n = 3) of mortality of *Mya arenaria* per *Cerebratulus lacteus* in function of predator density after 1, 2 and 3 weeks.

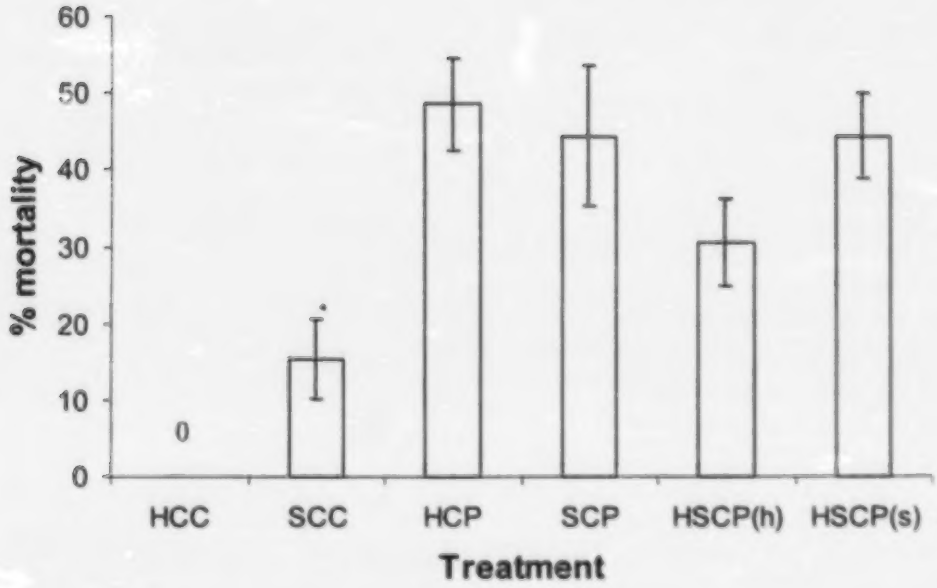

Figure 5. Percentage (mean ± SE; n = 3) of mortality of healthy and sick *Mya arenaria* in the presence of *Cerebratulus lacteus*. (HCC: healthy clam control; SCC: sick clam control; HCP: healthy clam with predator; SCP: sick clam with predator; HSCP(h): healthy and sick clams with predator (mortality of healthy clams); HSCP(s): healthy and sick clams with predator (mortality of sick clams))

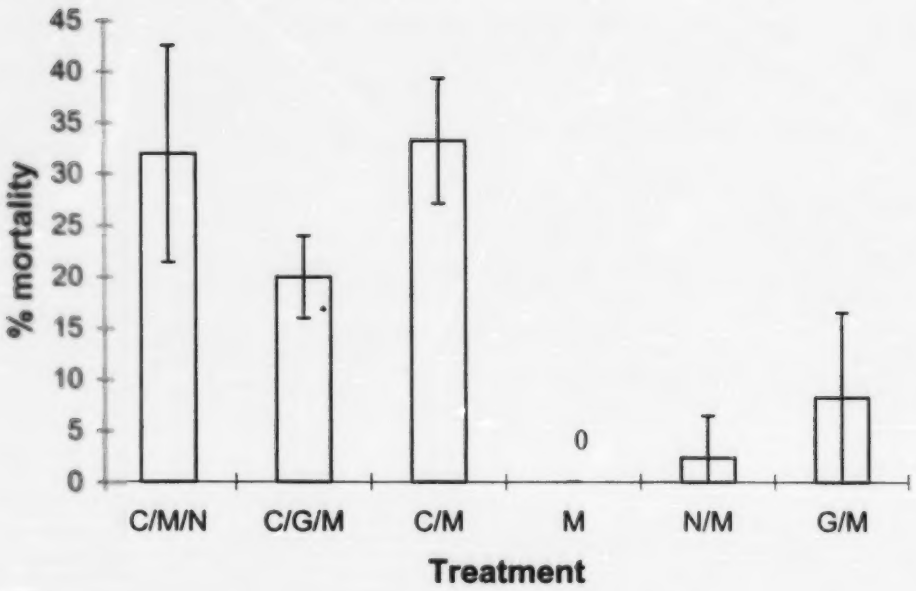

Figure 6. Percentage (mean ± SE; n = 3) of mortality of *Mya arenaria* using polychaetes as a control measure. (C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*; C/G/M: *C. lacteus*, *G. dibranchiata* and *M. arenaria*; C/M: *C. lacteus* and *M. arenaria*; M: *M. arenaria*; N/M: *N. virens* and *M. arenaria*; G/M: *G. dibranchiata* and *M. arenaria*)

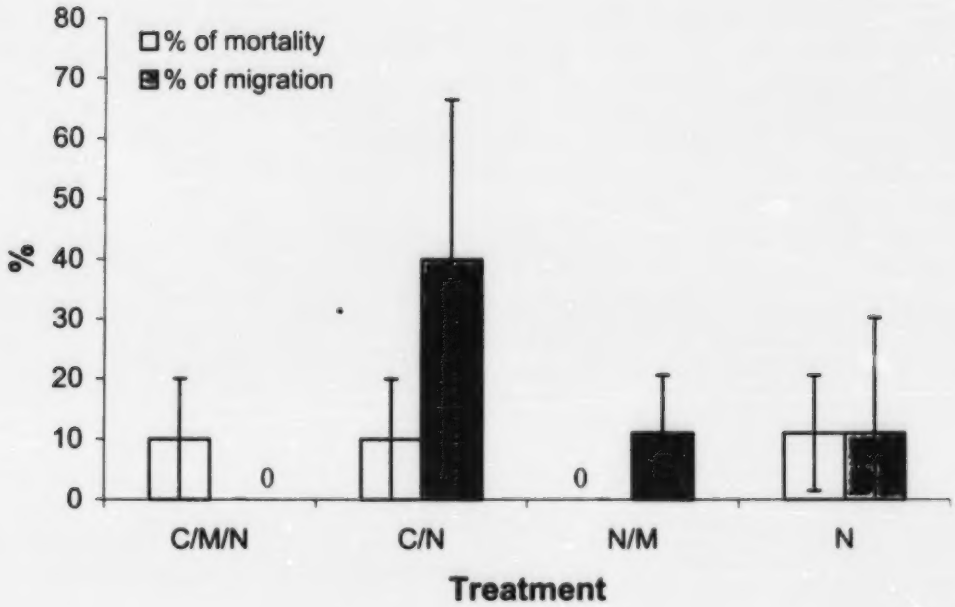

Figure 7. Percentage (mean ± SE; n = 3) of migration and mortality of *Nereis virens* in relation to different species combinations. (C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*; C/N: *C. lacteus* and *N. virens*; N/M: *N. virens* and *M. arenaria*; N: *N. virens*)

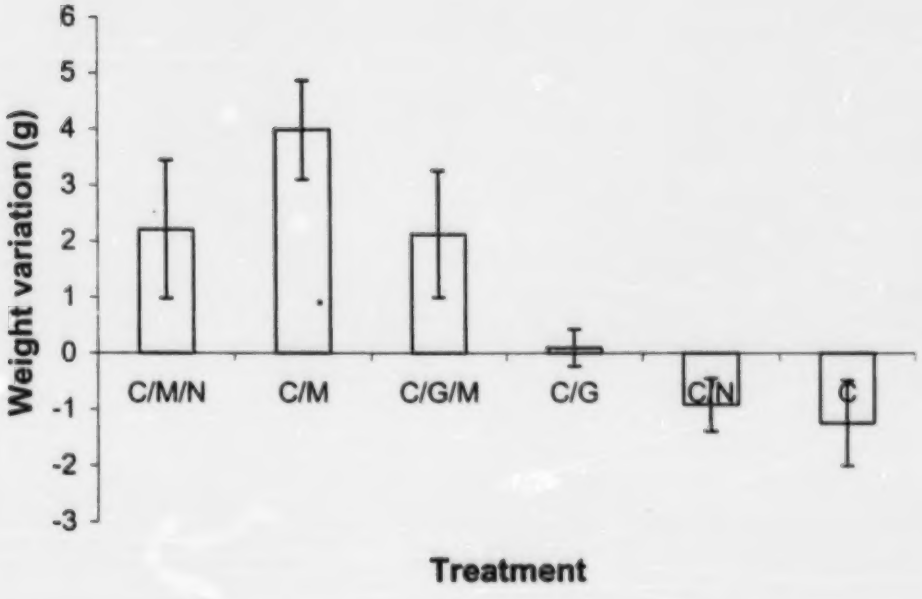

Figure 8. Weight variation (mean ± SE; n = 3) of *Cerebratulus lacteus* in polychaete addition experiment. (C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*; C/M: *C. lacteus* and *M. arenaria*; C/G/M: *C. lacteus*, *G. dibranchiata* and *M. arenaria*; C/G: *C. lacteus* and *G. dibranchiata*; C/N: *C. lacteus* and *N. virens*; C: *C. lacteus*)